THE BRANCHIAL CHAMBER IN TERRESTRIAL CRABS:
A COMPARATIVE STUDY

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Adaptive responses to different environments can be functional, structural, behavioral or a combination of these. The exposure to different environments can be due to changes in environmental factors or due to the invasion of new habitats by a progressive migration of organisms. Invasion of the land has required a number of such adaptations in amphibious crabs (Bliss, 1968), foremost among these being adaptations connected with respiratory mechanisms. Comparisons between aquatic and terrestrial crabs have shown that the latter have fewer gills (Pearse, 1929, 1950) and reduced gill surface per unit weight (Gray, 1957; Bliss, 1963). Also, the gills of terrestrial crabs have become highly sclerotized on the edges, presumably to keep them erect and functional in air (Harms, 1932). A connection has often been mentioned between the globose carapace of Gecarcinidae, Potamonidae, Pseudothelphusidae and Trichodactylidae and their ability for aerial respiration.

It has been suggested that, during the evolution towards a terrestrial habitat, the reduction of gills and gill area is compensated by an increasingly more important role in respiration played by the thin epithelial membrane that lines the branchial chambers (Carter, 1931; Edney, 1960). The lining of the gill chamber has been referred to by many authors as the "lung" of land crabs. Indeed, some indications of this "lung" exist in the vascularized epithelium of the branchial lining in *Uca* (Jobert, 1876) and *Cardisoma* and in the tufts arising from the branchial lining in species of *Ocypode, Coenobita* and *Birgus* (Borradaile, 1903; Harms, 1932).

Of the 4,500 species of brachyuran crabs so far known, many which live in intertidal zones can withstand various degrees of exposure to air. However, true amphibious life is restricted to a few terrestrial and freshwater forms, most of them belonging to the families Grapsidae, Gercarcinidae, Ocypodidae, Potamonidae, Trychodactylidae, Pseudothelphusidae, and Myctyridae (Bliss, 1968). In the present study, evidence is presented on the respiratory function of the lining of the branchial chamber in several semiterrestrial brachyuran species from marine, estuarine, and freshwater habitats.

MATERIALS AND METHODS

In the course of the present study, seven brachyuran species belonging to four families were used for estimates of branchial chamber volume, observations of the chamber's shape, and examination of the structure of the lining which covers the chamber. The sites of collection varied from the supralittoral zone of sandy beaches to the mountains of tropical forest and the llanos (savanna-like forest).

Material examined

Trychodactylidae: *Valdivia venezuelensis* Rathbun, *Dilocarcinus dentatus* (Randall). Type of habitat: savanna-like areas (llanos) in large rivers, ditches and ponds, either on the bottom or in deep holes in the banks of the rivers. These crabs are able to stand long periods of time out of the water (Holthuis, 1959; Valente, 1948). Site of collection: at rivers in the Venezuelan llanos (Calabozo, State of Guárico) at 150 m above sea level.

Pseudothelphusidae: *Eudaniella iturbei* (Rathbun), *Microthelphusa simoni* (Rathbun). Type of habitat: small mountain creeks and streams where they dig shallow burrows under large stones that are surrounded by water; these burrows might be extended 30 cm deep or more under the stream bed. Species of this group can live long periods of time out of the water and survive when small streams dry up during the dry season (Holthuis, 1959). Site of collection: *E. Iturbei* at small mountain creeks at 1,300 m above sea level in the Venezuelan coastal range (State of Miranda); *M. Simoni,* at same area, but only 40 m above sea level.

Gecarcinidae: *Gecarcinus lateralis* (Fréminville). Type of habitat: this species digs its burrows in dry sand high up on the beaches and dry sparse grassland where ground water is not close by and the only moisture available comes from infrequent rain showers or dew (Bliss, 1963). Site of collection: supralittoral zone of sandy beaches at the central coast of Venezuela (State of Miranda).

Cardisoma guanhumi Latreille. Type of habitat: this species inhabits low-lying areas up to 8 km away from the sea. This crab is commonly found in mangrove swamps, where it digs down to the ground water (Herreid and Gifford, 1963). This species is capable of surviving long periods of submersion in water (Gifford, 1962). Ovigerous females of *G. lateralis* and *C. guanhumi* usually migrate into the sea for spawning. Site of collection: mangrove swamps at the central coast of Venezuela (State of Miranda).

Ocypodidae: *Ocypode quandrata* Fabricius. Type of habitat: this crab is commonly found in the supralittoral zone of sandy beaches where it digs deep holes distinctively located from just above high tide mark to some 500 m into the dunes (Bliss, 1968). Adult specimens of this species are nocturnally active, but juveniles may be seen out of their burrows during the day (Hughes, 1966). Site of collection: supralittoral zone of sandy beaches, coast of Venezuela (State of Miranda).

The volume of the branchial chamber was measured in at least 32 specimens of each species (except in *Eudaniella iturbei,* where only 14 specimens were available); and, at the same time, body weight, carapace width, and carapace length were recorded. The specimens were killed by immersion in 10% formalin and weighed after removal of surplus water from the body and gill chamber with a paper towel and removal of the pereiopods. The pereiopods were amputated in order to avoid possible variability in weight due to total loss or regeneration of some limbs prior to collection. To determine the volume of the branchial chamber, melted paraffin was perfused through the efferent channels, completely filling the chamber. The cast thus obtained showed the shape of the chamber, and its volume was determined from the weight and density of the paraffin cast. Since values for both chambers were obtained, a mean value was taken for each specimen.

The general morphology of the branchial chamber of several Antillian and Central and South American species of Pseudothelphusidae was studied. *Ucides cordatus,* a gecarcinid, was also cursorily examined. The lining of the branchial chamber in all species that were studied was routinely examined.

In several pseudothelphusid species, a new respiratory structure was discovered (see results). In order to determine the kind of relationship between the size of the animal (expressed as carapace breadth) and the morphological characteristics of the respiratory structure, ten specimens of *Eudaniella iturbei,* which spanned the size range of the species, were selected. Measurements were made of the diameter

of perforations found in the structure, its thickness, and the external area of the respiratory region; finally, the total number of perforations on the structure were estimated. Similar measurements were made on the other pseudothelphusid species (Table I).

To determine the localization of respiratory sites, a technique was used similar to that of Bertolini (1933, 1934) for holothurians, and Edney and Spencer (1955) for isopods. A solution of methylene blue (1.5 mg/ml, approximately) was prepared in boiled distilled water and then reduced by addition of a saturated solution of hydroquinone and sodium sulfite, hydroquinone alone, or sodium sulfite alone, until all blue color disappeared. From 1 to 5 μl of this reduced solution were injected every four hours into living crabs through the branchial veins, through the coxopodite of each pereiopod, or by direct puncture to the heart. Reduced methylene blue recovers its original color by oxidation and stains the tissues dark blue or black where oxygen uptake occurs. Crabs were sacrificed at different times after injection and the entire branchial chambers were inspected. In order to accelerate the process, some crabs were maintained in an atmosphere of nitrogen, and nitrogen was also forced into the gill chamber through the efferent channels. The animals were kept in this condition from 5 to 10 minutes, after which the heart was injected with reduced methylene blue. Immediately after injection, oxygen was forced into the branchial chamber, and the animals were maintained in an atmosphere of oxygen for not less than 15 minutes; then they were sacrificed.

Another set of specimens, not less than five per species, was used for histological study. Segments of the lining or the whole branchial chamber were fixed in Helly-Zenker solution (except for one instance, in which trichloroacetic acid was used) and stained with hematoxylin-eosin. Staining tests for the identification of mucopolysaccharides were carried out by the Periodic-Acid-Schiff (PAS) reaction, PAS inhibited by dimedone, and PAS inhibited by ptyalin (Pearse, 1960).

RESULTS

General morphology of the branchial chamber

The paraffin casts gave a three-dimensional view of the branchial chambers, as seen from the outside. In each case a dorsal, a frontal, and a lateral view of the left chamber is given (Figs. 1 and 2). The frontal portion of the crab corresponds to the upper part of the drawing in dorsal view and to the right-hand side of the drawing in lateral view. In the case of the frontal view, the left-hand side of the drawing corresponds to the lateral portion of the crab. In each view, the peripheral line of the drawing gives an approximate idea of the cross section of the chamber through each corresponding plane. The depressions shown on the surface of the drawing of the casts correspond to internal expansions and projections of the chamber lining.

Both *Cardisoma guanhumi* and *Gecarcinus lateralis* have a globular chamber, very simple in outline and without internal projections (Fig. 1, a to f). The gill chamber of *Ucides cordatus* presents the same general form; but at its lower part, just over the gills, the lining forms a fold that practically separates the gills from the rest of the chamber. In contrast to these species, *Ocypode quadrata* has a
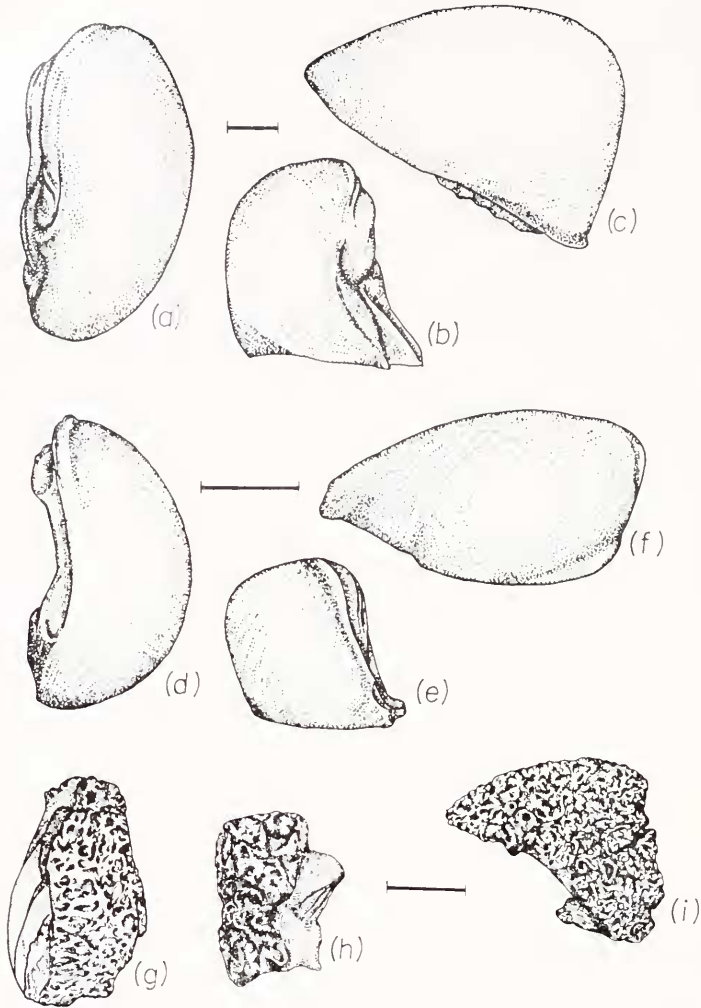
Figure 1. Shape of the lefthand branchial chamber, according to the paraffin casts obtained from different species. *Cardisoma guanhumi* Latreille: a) dorsal view; b) frontal view; c) lateral view. *Gecarcinus lateralis* (Fréminville): d) dorsal view; e) frontal view; f) lateral view. *Ocypode quadrata* (Fabricius): g) dorsal view; h) frontal view; i) lateral view. Casts for drawings were selected among those obtained from the largest specimens collected. Scale bars represent 1 cm.

pyramidal chamber (Fig. 1, g to i) with very conspicuous digitations and projections all over the upper, lateral, and anterior sides of the lining. In this case, a fold similar to that shown by *U. cordatus* is also present.

The chamber of *Dilocarcinus dentatus* is somewhat similar in shape to that of *O. quadrata,* but its wall is devoid of any kind of projections; however, several folds can be detected in the floor of the chamber (Fig. 2, a to c). Neither projec-

tions nor protuberances were observed in the lining of the gill chamber of *Valdivia venezuelensis*. In this case, the chamber was found to be similar in shape to that of *D. dentatus*. The few casts obtained from specimens of *V. venezuelensis* showed the frontal and posterointernal portions reduced by the ripe gonads.

In *Eudaniella iturbei* the branchial chamber is very much reduced in the posterior portion, the chamber being restricted to a globular cavity in the anterior half. In a mature female, the dorsal plane might be reduced anteriorly by development of the gonads along the frontal area of the specimen (Fig. 2, d to f). This general description of the branchial chamber of *E. iturbei* is also valid for *Microthelphusa simoni*.
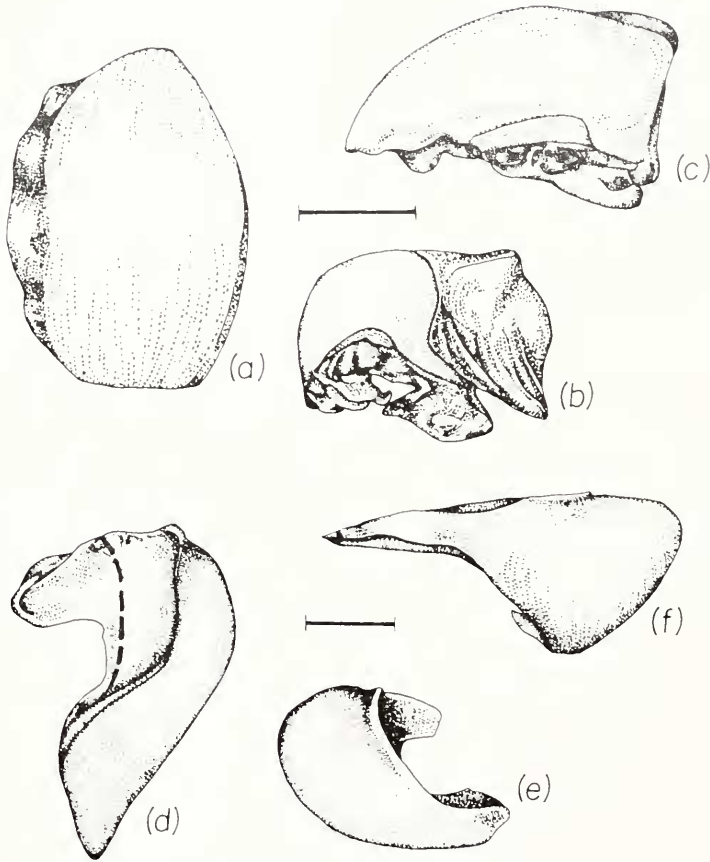

FIGURE 2. Shape of the lefthand branchial chamber, according to the paraffin casts obtained from different species. *Dilocarcinus dentatus* (Randall): a) dorsal view; b) frontal view; c) lateral view. *Eudaniella iturbei* (Rathbun): d) casts show the effect of gonad expansion into the lumen of the branchial chamber; dashed line indicates the normal outline of the chamber in the dorsal view; e) frontal view; f) lateral view. Casts for drawing were selected from those obtained from the largest specimens collected. Scale bars represent 1 cm.
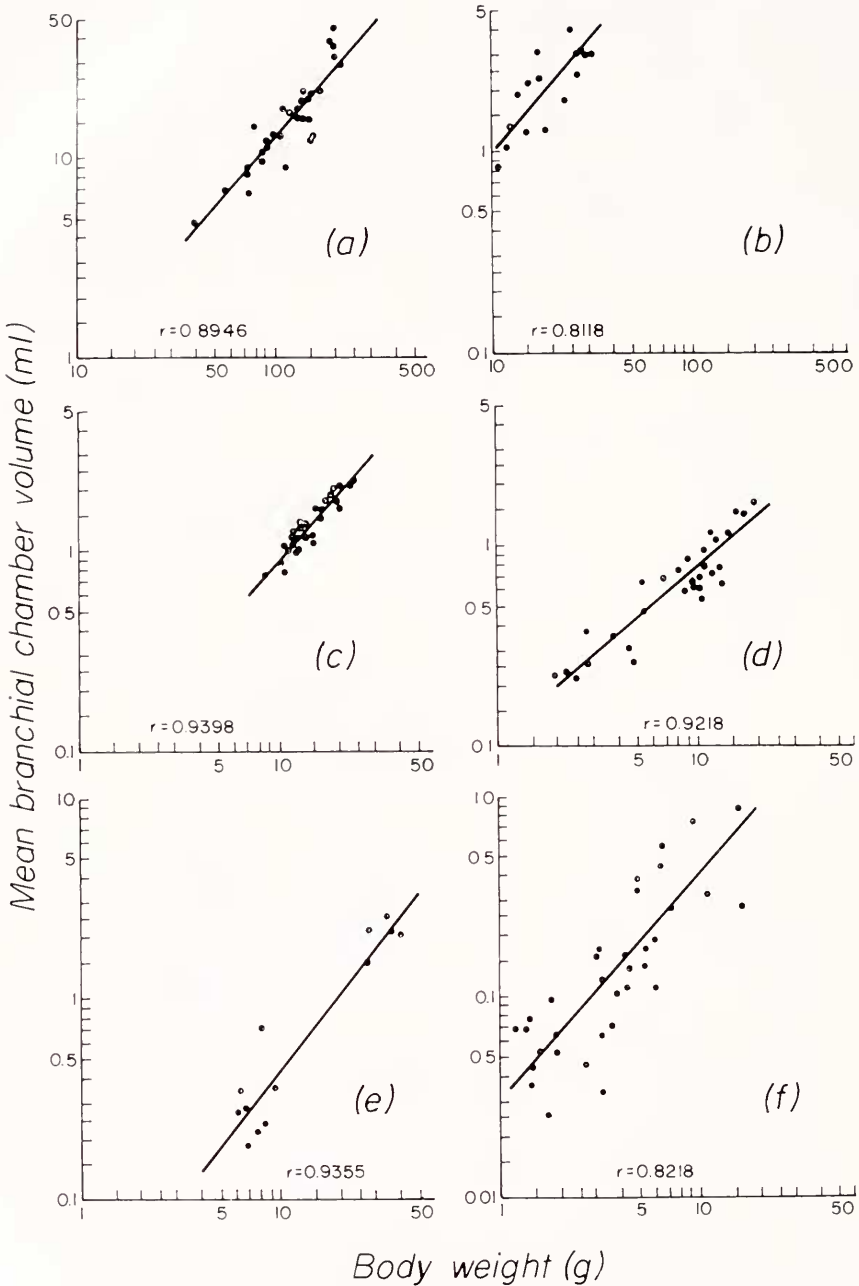
FIGURE 3.   Data points and regression lines expressing the relationship between weight of the crab's body without pereiopods and the mean volume of its branchial chamber: a) *Cardisoma guanhumi*, $y = 0.0614x^{1.158}$; b) *Gecarcinus lateralis*, $y = 0.0786x^{1.145}$; c) *Dilocarcinus dentatus*, $y = 0.0636x^{1.135}$; d) *Ocypode quadrata*, $y = 0.1062x^{0.862}$; e) *Eudaniella iturbei*, $y = 0.0235x^{1.272}$;

Relative growth of the branchial chamber

Regression lines were obtained by relating the weight of the crab's body without pereiopods (x) and the branchial chamber volume (y) (Fig. 3). For all these cases, a linear equation expresses the relationship between the logarithms of the two variables, with a correlation coefficient greater than 0.81 $(P < 0.01)$; thus, an allometric growth of the branchial chamber was assumed for all the species considered in this section. Bartlett's method was used to determine the relationship between logarithms (Simpson, Roe and Lewontin, 1960). Comparisons were made among the six species to test for differences between each pair of values of slopes and also for the values of y, when x = 1. These tests revealed that the slopes were not significantly different $(P < 0.05)$. However, comparisons made among the points of intersection suggested that there were three categories of branchial chamber development. A group with a relatively large branchial chamber was represented by *C. guanhumi, G. lateralis,* and the river crab *D. dentatus.* A group with a relatively small branchial chamber was formed by the mountain freshwater crabs, *E. iturbei* and *Microthelphusa simoni;* and an intermediate branchial chamber group was represented by *O. quadrata.*

The regression lines obtained for the species forming each of the extreme groups did not show any significant differences $(P < 0.05$ with respect to slope and intersection on the y-axis. Each group or category proved to differ significantly $(P < 0.05$ from the other two only in the y-intersection.

The perforated area or "lung"

All the species of Pseudothelphusidae examined show a distinctive perforated area located on the ceiling of the branchial chamber wall over both anteroexternal angles of the body. The general illustration given in Figure 4 is for *Eudaniella iturbei,* but similar structures were observed in all pseudothelphusids from Venezuela, as well as in all of the specimens from Central and South America and the Antilles. *Potamocarcinus mociñoi,* a Mexican cave dwelling pseudothelphusid, was the only species examined that did not show this characteristic perforated area. The lining of the branchial chamber was devoid of any kind of specialized structure.

The perforated area is formed by small holes closely placed in an oval distribution. The area of the perforated zone, the density of perforations, the mean diameter of the channels, and the thickness of the structure are related to the size of the animals and to the species considered. Table 1 shows the values obtained for several parameters measured on the perforated area of several species of Pseudothelphusidae.

Unfortunately, there were not enough available specimens of some species to confidently establish interspecific comparisons. However, the general trend of variation of the characters measured was obtained for one species. Figure 5 shows the data points and the respective regression lines that establish the relationships between the carapace breadth of *Eudaniella iturbei* and the measured characteristics of the perforated area found in the lining of its branchial chamber. For this species,

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and f) *Microthelphusa simoni,* $y = 0.0311x^{1.140}$. The correlation coefficient for each of the regression lines is given.
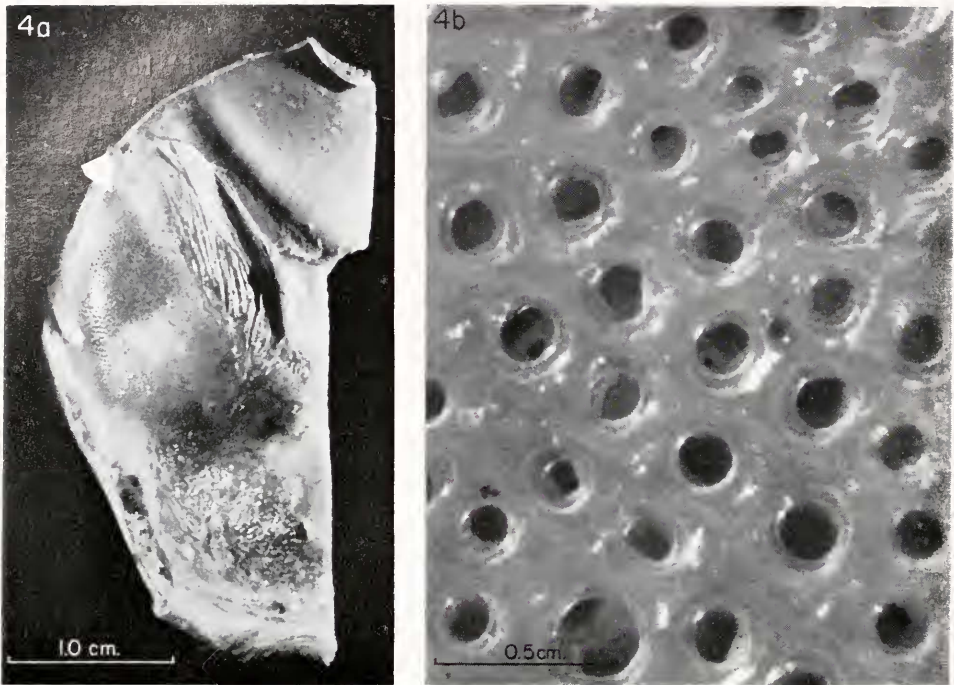
FIGURE 4. *Eudaniella iturbei* (Rathbun) : a) ceiling of the branchial chamber; and b) detail of the exterior surface of the perforated area found in the ceiling (anterolateral angle) of the branchial chamber.

the data revealed linear relationships between the mean diameter of the holes and the carapace breadth (x) (Fig. 5a), as well as the thickness of the structure and the carapace breadth (Fig. 5b). A negative power equation ($y = bx^{-c}$) was determined for the regression line obtained when comparing the carapace breadth with the estimated mean number of holes per mm^2 (Fig. 5c). In the case of the external area of the perforated region (y), a power equation appeared to be the best fit (Fig. 5d). A correlation coefficient above 0.94 was found for all these cases, being significant at 0.01 level. Using the estimated density of perforations and the total area of the structure considered, an estimate was obtained for the total number of perforations. There was no significant relationship ($P > 0.05$) between the size of the animal and the total estimated number of perforations.

Sites of oxygen uptake

Assays made with reduced methylene blue gave similar results, regardless of the reduction agent used. In all cases the branchial lamellae of the specimens were stained dark-blue or black within five to 24 hours. The process was accelerated (10–15 min) when the crabs were kept for a few minutes in air deprived of oxygen, injected with methylene blue, and subsequently transferred to an atmosphere rich in C_2. Besides the staining of the lamellae, in *Cardisoma guanhumi* and *Gecarcinus lateralis*, the entire lining of the branchial chamber was also stained in addition to

TABLE I

Dimensions of the "lung" found in several species of Pseudothelphusidae.

Species and localities of collection	Specimen number	Carapace breadth (mm)	Mean diameter perforations (mm)	Mean number perf./mm²	Mean thickness (mm)
Pseudothelphusa dugesi belliana	1	24.3	0.0513	29.15	1.0064
Rathbun, El Serpentin,	2	36.0	0.1071	20.36	0.7850
Michoacan State, Mexico	3	41.7	0.1087	13.04	1.5097
Pseudothelphusa dugesi dugesi	1	25.0	0.0701	32.87	—
Rathbun, Chignahuapan,	2	38.6	0.0796	23.75	1.1474
Puebla State, Mexico					
Pseudothelphusa jouyi Rathbun	1	30.3	0.0513	12.90	0.2738
Camecuaro, Michoacan					
State, Mexico					

Perforations ($\phi = 0.05$ mm) were observed over the entire chamber's lining. An inconspicuous "lung" was found from which measurements were made.

Pseudothelphusa dilatata	1	44.0	0.0993	29.47	1.5097
Rathbun, Las Estacas, Morelos	2	45.5	0.1047	25.21	1.0266
State, Mexico					

A well formed "lung" was observed, accompanied by perforations ($\phi = 0.0797$) scattered (9.71 mm²) over the chamber lining.

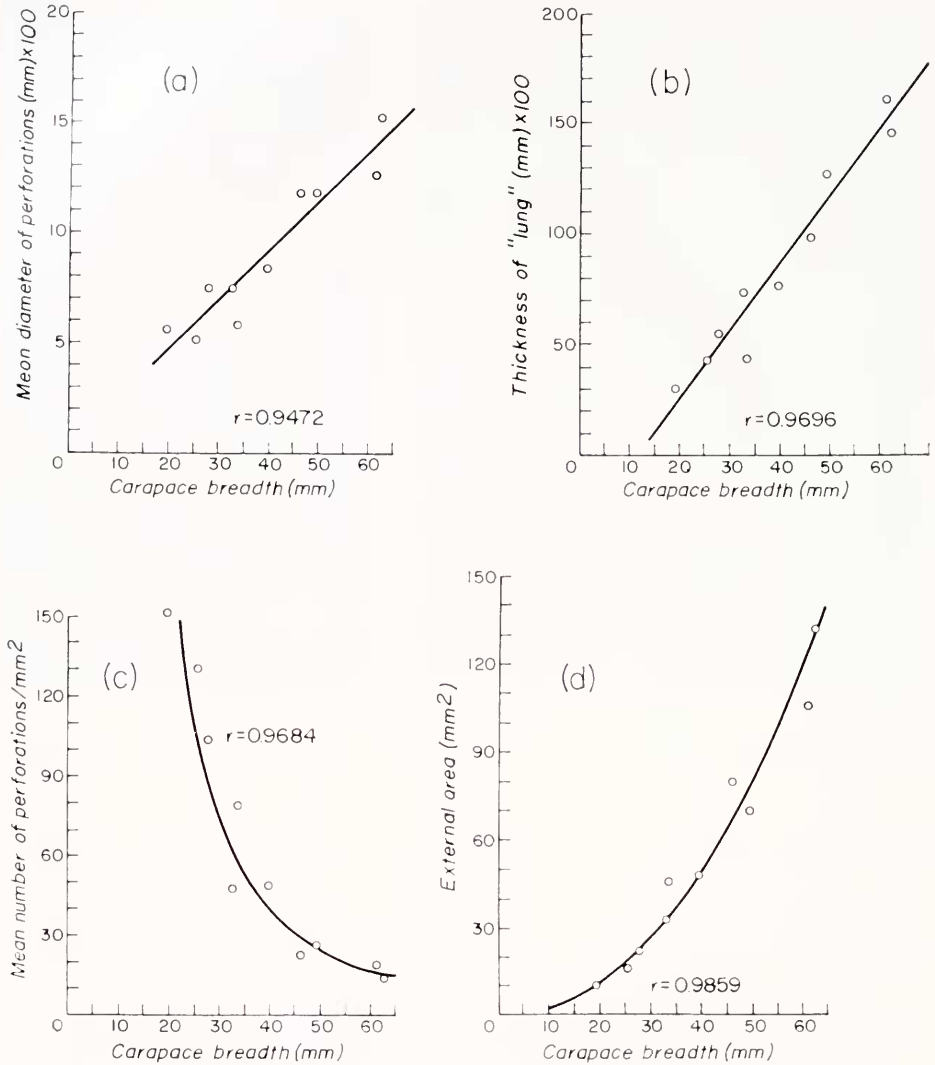|					
Neopseudothelphusa fossor	1	29.7	0.0900	33.88	0.8639
(Rathbun) Chupsa, Miranda	2	26.8	0.0653	32.24	0.5667
State, Venezuela					
Eudaniella garmani (Rathbun)	1	82.9	0.2317	8.12	2.4000
Rio Nuevo, Sucre State,					
Venezuela					
Eudaniella ranchograndensis	1	41.0	0.0740	37.58	0.8166
(Rodríguez) Limón River,					
Miranda State, Venezuela					
Potamocarcinus maxillipes	1	68.1	0.1248	8.77	1.2882
(Rathbun) Teapa, Tabasco					
State, Mexico					
Potamocarcinus mociñoi (Rioja)		Three specimens were examined, none of which presented			
Cuevas del Tio Ticho, Comitán,		any conspicuous structure that resembled the "lung"			
Chiapas State, Mexico		commonly found in the other species. The lining was			
		smooth and thin without any folds or protuberances.			
Potamorcarcinus armatus Milne	1	67.1	0.0860	11.32	1.3666
Edwards, Near Granada,					
Gran Lago, Nicaragua					
Epilobocera sinuatifrons (A.	1	18.6	0.0316	245.15	0.2233
Milne Edwards) El Yunque,	2	73.5	0.1232	11.61	1.5040
Luquillo and near Mayaguez,	3	80.8	0.1433	9.05	1.5733
Puerto Rico	4	97.1	0.1548	7.45	2.9330
Guinotia dentata (Latreille)	1	62.0	0.1469	13.14	1.2220
Syndicate Road, Dominica					

FIGURE 5. *Eudaniella iturbei* (Rathbun). Data points and regression lines express the relationships between carapace breadth (taken as expression of size of the animal) and several characters measured from the "lung" located in the lining of the branchial chamber: a) diameter of the perforation, $y = 0.2043 + 0.2236x$; b) thickness of the structure, $y = -34.632 + 3.029x$; c) number of perforations per mm², $y = 92276.57x^{-2.0962}$; and d) total external area estimated for the perforated structure, $y = 0.0186x^{-2.1433}$. The correlation coefficient for each of the regression lines is given.

the branchial lamellae. In the pseudothelphusids, *Eudaniella iturbei* and *Neopseudothelphusa fossor*, only the branchial lamellae and the perforated area of the chamber's lining were stained; the chamber's lining itself was not stained. Similarly, in *Ocypode quadrata* only the lining's folds were stained in addition to the gills.

Structure of the branchial chamber wall

In *Ocypode quadrata,* as in other species studied, a section normal to the carapace surface (Fig. 6, a to c) showed a thin external layer of connective tissue, with groups of amber-colored melanin granules, corresponding to the chromatophores. The middle layer was a spongy tissue, with numerous blood vessels branching off towards the lumen of the chamber, and the internal layer was an epithelium composed of a simple cell layer. In the digitations and projections, thin blood vessels were particularly abundant, and numerous nonstriated muscle fibers were observed to reach almost to the internal epithelium (Fig. 6c). There were numerous birefringent spheres throughout the wall and particularly in the digitations, usually eosinophilic but sometimes basophilic in character. Such spheres possessed a PAS-positive substance, and their content sometimes showed a granular appearance. In these instances, the contour became less distinct, and spheres seemed to empty into the stroma of the tissue (Fig. 6c).

In *Cardisoma guanhumi* the layer of spongy tissue immediately below the chromatophore layer (Fig. 7a) had groups of cells organized in a pattern similar to a glandular tissue (Fig. 7, b to e). There appeared to be different stages of organization, either as groups of closely packed cells radiating from the center, or disposed as a thin-walled glandular structure whose lumen was filled with granular eosinophile substance (Fig. 7, b to e). For the rest, the branchial chamber wall had large blood vessels, and some nonstriated muscular fibers arranged tangentially and transversely wrapped in sheets of connective tissue. The internal layer was a thin epithelium (Fig. 7f). The structure of the wall in *Gecarcinus lateralis* (Fig. 8, a to d) was similar to that of *Cardisoma guanhumi,* but the gland-like structures were not observed.

The three layer structure was also present in the Pseudothelphusidae (Fig. 9a, b). Large birefringent PAS-positive spheres and a few fibers of nonstriated muscle were observed in the middle layer of the peripheral area of the perforated region on the lining or "lung" (Fig. 9b). The "lung" presented numerous channels or perforations, more or less straight in longitudinal section (Fig. 9a), but of irregular shape in cross section (Fig. 9d). The walls of these channels possessed folds (Fig. 9a, c) that sometimes gave rise to anastomosis between the channels.

The tissue among the channels was the characteristic spongy tissue of the middle layer, with numerous blood vessels and sinuses (Fig. 9, c to e). The internal side of the channel was lined with the usual one-celled epithelium found in the rest of the branchial chamber walls examined.

Valdivia venezuelensis and *Dilocarcinus dentatus* showed the basic structure of other species (Figs. 10 and 11), with an external layer with melanin granules, a middle layer of spongy tissue with numerous blood vessels and sinuses, and an internal one-celled epithelium. In the middle layer there were conspicuous birefringent PAS-positive spheres (Figs. 10 and 11) and fibers of nonstriated muscle covered by a sheet of connective fibers, arranged in a longitudinal and transverse pattern. In *Valdivia venezuelensis*, the nonstriated fibers crossed the wall from the external to the internal sides (Fig. 10d). In addition, this species possessed striated fibers, more numerous on the lateral sides of the chamber. Groups of cells with large nuclei were observed at the insertion point of these striated muscular fibers (Fig. 10b, c).
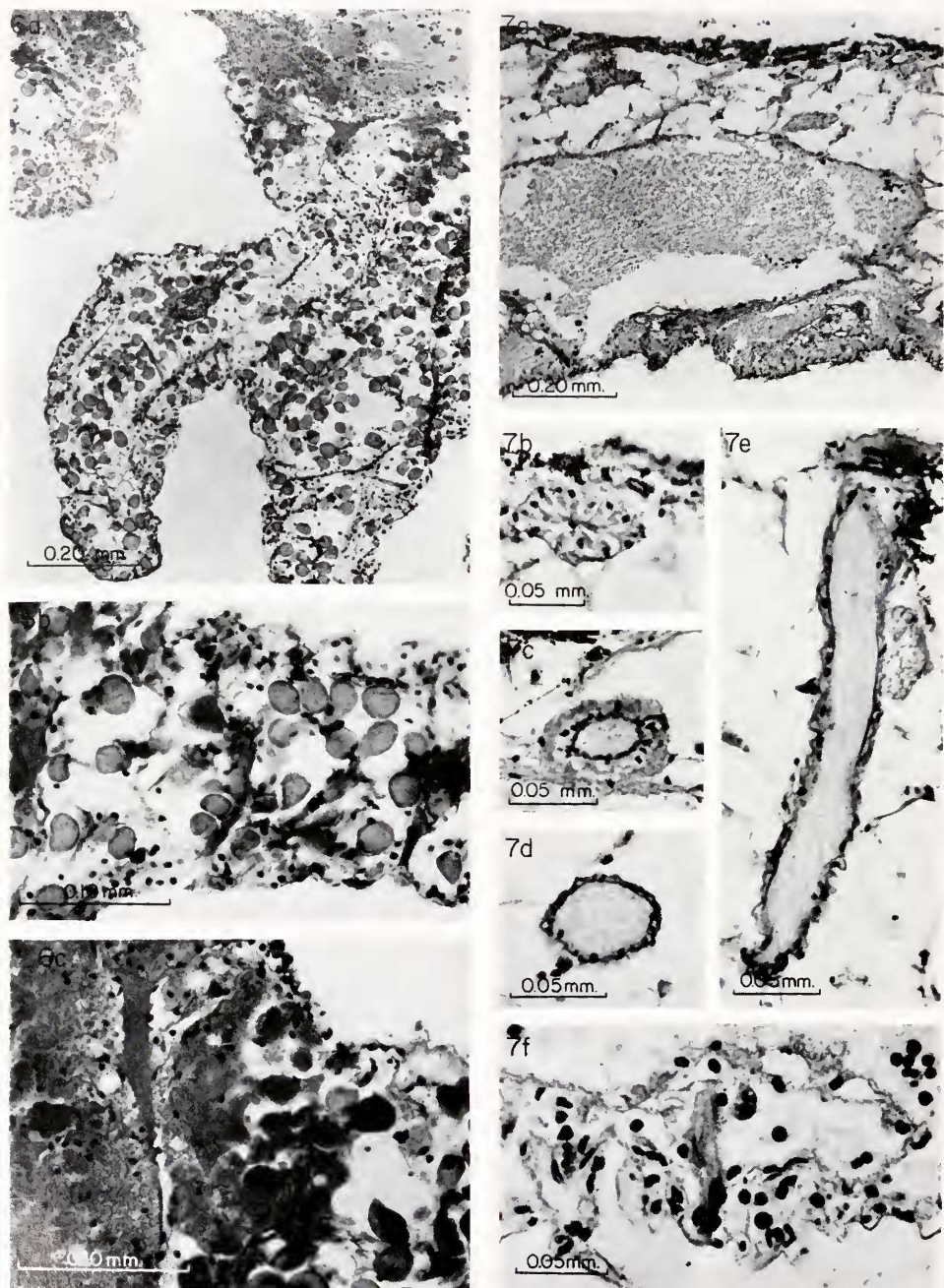
FIGURE 6. *Ocypode quadrata* (Fabricius) : a) low power view of a transverse section of the branchial chamber lining; b) view at higher magnification of the middle area of the transverse section of a fold; c) view of the tissue showing the granular aspect of some birefringent

Discussion

Certain morphological characteristics of land crabs have been seen as expressions of adaptation to their habitats. Generally, the more terrestrial forms have fewer gills and a reduced gill area when compared to more aquatic groups, regardless of their phylogenetic relationship (Gray, 1957; Veerannan, 1974). In general, land crabs have a large branchial chamber which is very vascularized and may contain folds (Edney, 1960). These characteristics have been used by several authors as indications of a "lung" in amphibious crabs, (Jobert, 1876; Borradaile, 1903; Harms, 1932; Carter, 1931; Zoond and Charles, 1931).

The suggested respiratory function of the chamber's lining in amphibious crabs was confirmed by the oxidation of methylene blue for all the species tested. Furthermore, the staining was restricted to the gill lamellae and particular areas of the chamber's lining, *i.e.,* the perforated area of *Eudaniella iturbei* and *Micro-thelphusa simoni,* and the folds of the chamber's lining in *Ocypode quadrata.* Although the methylene blue test was carried out in only two pseudothelphusid species, we believe the results can be extrapolated to the other pseudothelphusids examined, with the exception of *Potamocarcinus mociñoi.* This was the only species examined which did not show the perforated area.

The lamellae of the gills of terrestrial crabs appear to be strengthened with chitinous ridges, which presumably keep them erect and functional in air (Harms, 1932). The air flow inside the branchial chamber apparently occurs by the beatings of the scaphognatite (Cameron, 1975), which provides the oxygen taken by diffusion through the existing water film on the lamellae (Valente, 1948). However, Standaert (1970, p. 79), working with *Cardisoma guanhumi,* found that "the gill lamellae stack together and fill with capillary water and appear inefficient for gas exchange." Thus, there is no guarantee of an efficient air flow between the gill lamellae. Furthermore, Cameron (1975) found no evidence for an efficient air flow in the gill chamber of *C. guanhumi* and *Gecarcinus lateralis.* These findings suggest the need for an accessory surface for oxygen uptake, such as that determined by the methylene blue test in both species.

Supporting evidence for the respiratory function detected in the branchial chamber's lining is provided from the study of the structure of the chamber's wall. The spongy aspect of the intermediate layer of the lining and the profusion of blood vessels and sinuses already suggest a respiratory function. Such characteristics of the lining's tissue are common to all species examined. However, in the absence of additional studies, it is difficult to assign a role to some structures found within the chamber's wall. The function of the globules of mucopolysaccharide observed in *Ocypode quadrata* and the freshwater crabs is to be investigated. It can be speculated that the muscular fibers found in all the species studied could be used in contracting the wall and thus facilitating the circulation of blood in the sinuses.

spheres and a large blood vessel. The lumen of the branchial chamber corresponds to the upper-right corner of the figure.

FIGURE 7. *Cardisoma guanhumi* Latreille: a) general view of a transverse section of the branchial chamber lining; b), c) and d) transverse sections of glandular structures from the upper layer of the lining of the branchial chamber; e) longitudinal section of a similar structure from the same region; and f) view at higher magnification of the tissue surrounding the lumen of the chamber.
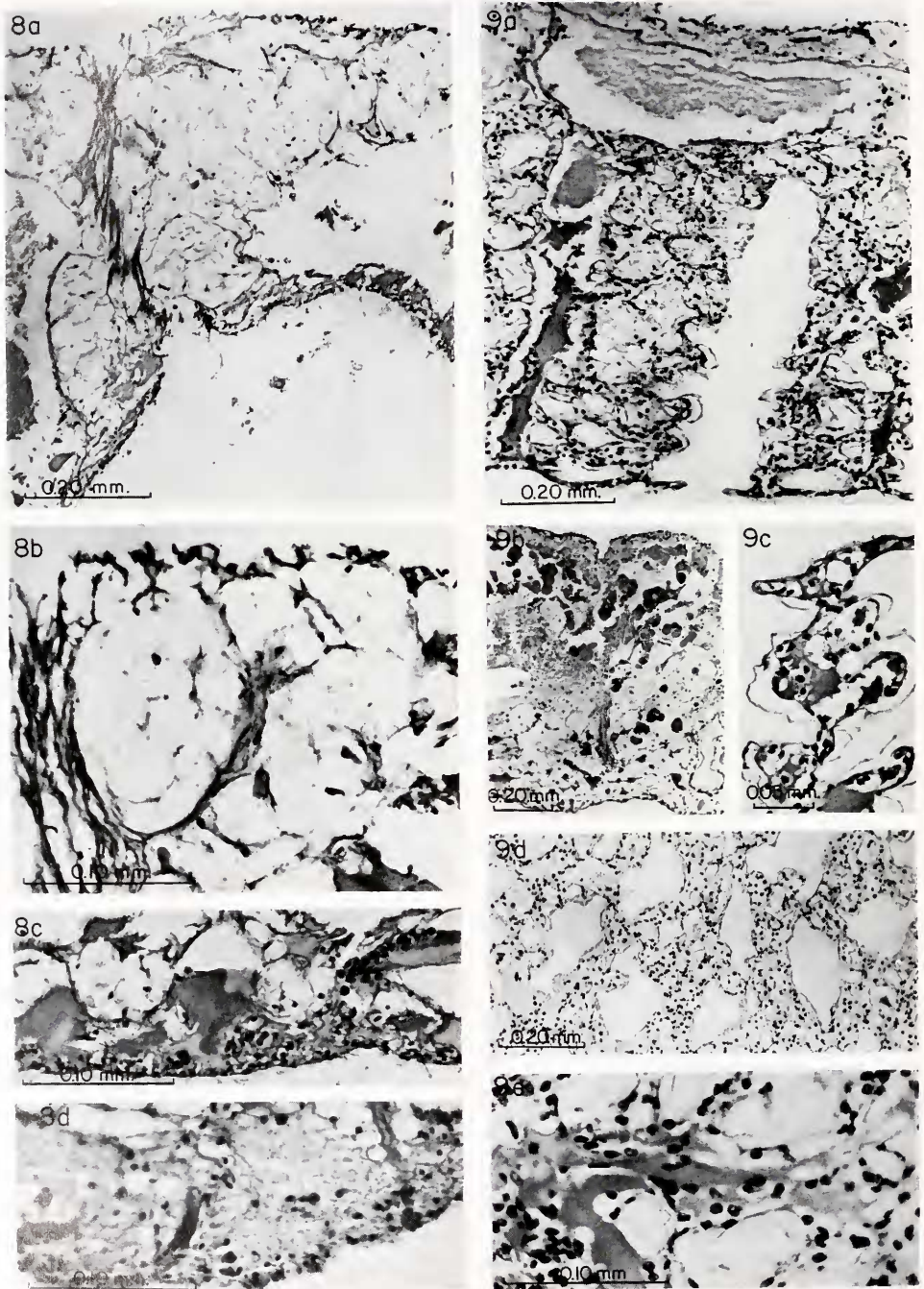
FIGURE 8. *Gecarcinus lateralis* (Fréminville): a) low power view of a transverse section of the branchial chamber lining; b) view of higher magnification of the upper layer of the

No indication exists of the possible function of the gland-like structures found in *Cardisoma guanhumi,* unless these structures were related to the molting process, *i.e.,* the tegumental glands referred to by Dennell (1960).

Respiratory surface additional to the gill lamellae is provided by the folds in *O. quadrata.* A more efficient way for increasing surface without sacrificing space is the perforations or respiratory channels presented in the "lung" of pseudothelphusids, especially when the anastomosis of those channels, shown by sections of *Eudaniella iturbei* and *Microthelphusa simoni,* is considered.

The data collected from *Eudaniella iturbei* give some indications about the development of the perforated structure during the body growth in this species (Fig. 5, a to d). The area increases exponentially with size, while the density of perforations decreases in a similar fashion. Since the diameter of the holes increases only linearly with size of the animal, it would be reasonable to expect a similar number of perforations for the whole size range of the species. It is, therefore, not surprising that no significant relationship was obtained between the size of the animal and the estimated total number of perforations in the structure.

As the animal grows and its metabolic requirements increase, a larger surface for O_2 uptake would be needed. However, additional surface area would not have to be created by new external perforations in the structure. In this case, it appears to be produced by anastomosis of the channels in the original structure. The increased anastomosis would correspond to the linear increments in thickness of the "lung" (Fig. 5b). Data collected from other species (Table I) seem to indicate a differential increase in respiratory surface of the "lung" in relation to the species considered. The "lung" has several degrees of complexity, which appear to agree with the proposed evolutionary trend of Pseudothelphusidae (Rodríguez and Smalley, 1969). The complexity of the "lung" increased from one species to the other: it was absent in *Potamocarsinus mociñoi,* was simple and accompanied by perforations in the lining in *Pseudothelphusa jouyi,* was thicker in *P. dilatata,* and was complex in all other species which had no additional perforations in the chamber's lining (see Table I). In this latter category, the mean diameter of perforations, mean number of perforations per mm^2 and mean thickness of the "lung" were variable (Table I). However, due to lack of information for the whole size range of each species, a confident comparison among species could not be reached nor could the evolution of the "lung" in Pseudothelphusidae be traced.

The examination of the casts obtained with paraffin from the branchial chamber indicated several types of modifications of the gill chamber's shape, from the globose form found in Gecarcinidae to the pyramidal form of the Tricodactylidae. A simple lining was found in both families. A pyramidal gill chamber was also found in *Ocypode,* but with numerous folds in its lining. Pseudothelphusidae presented an elongated chamber with a globose portion in the anterior half, accom-

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branchial chamber lining; c) and d) high power view of the lower layer of lining surrounding the chamber's lumen.

Figure 9. *Eudaniella iturbei* (Rathbun) : a) low power view of a transverse section of the branchial chamber wall showing the lumen of one perforation; b) low power view of a transverse section of the chamber's wall from an area different from that where the "lung" is normally found; c) high power view of the tissue which forms the perforations; d) and e) view at high magnification of longitudinal sections of the "lung" of the chamber's lining.
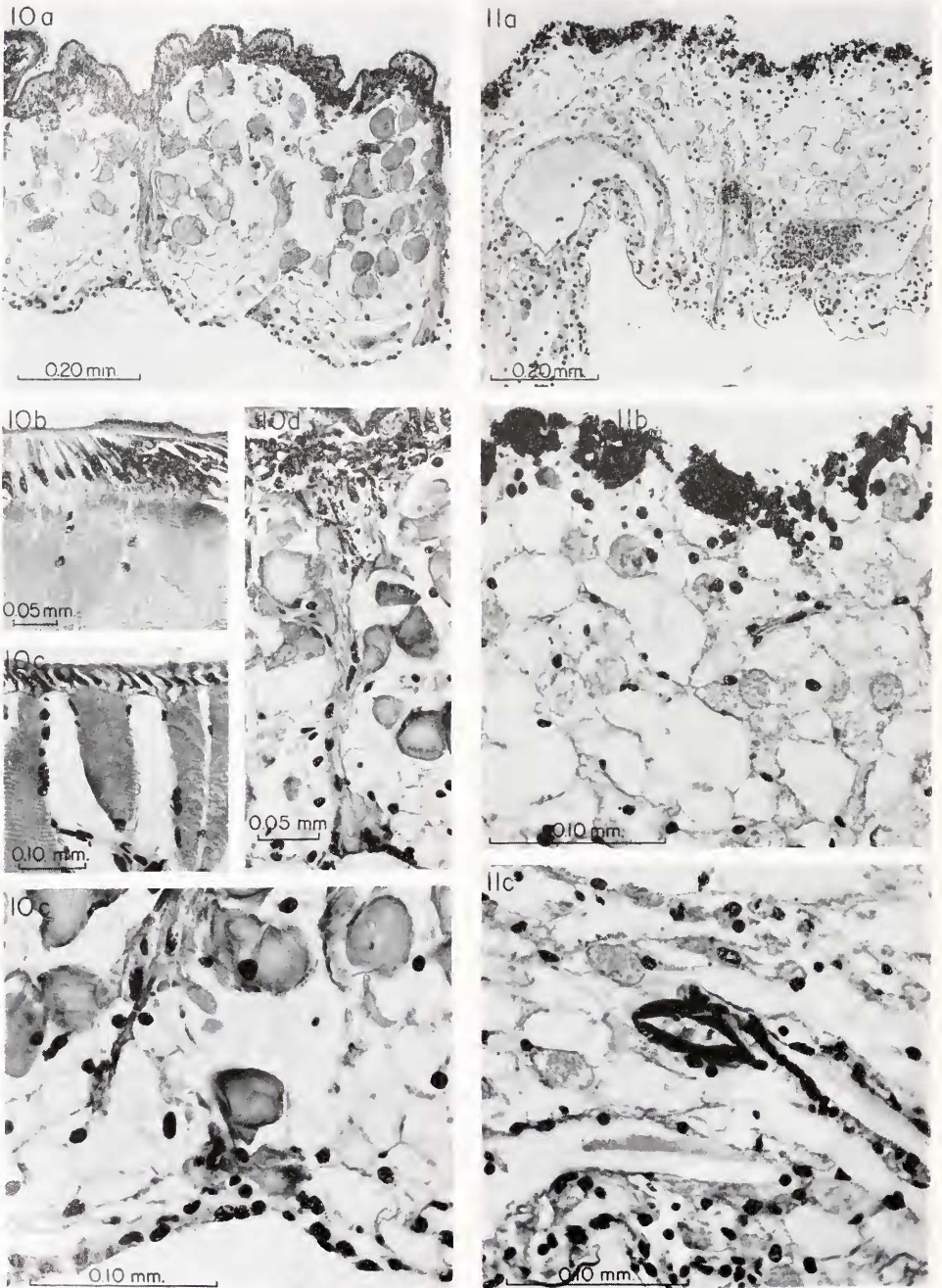
FIGURE 10. *Valdivia venezuelensis* Rathbun: a) low power view of a transverse section of the branchial chamber wall; b) and c) from the chamber's sides, high power views of the upper layer of the branchial lining, showing insertions of muscular and connective tissue; d)

panied by the development of the "lung" described in the present work. The changes in shape of the gill chamber do not seem to be connected with the habitat of the species, but rather with the development of larger respiratory surfaces, accessory to the branchial system.

The enlargement of the branchial chamber and modifications in the structure of the epithelium covering the inner surface of the chamber, such as more abundant vascularization and increment of surface area by fold and tufts, have been interpreted as a general trend in land crabs for adaptation to life in air (Wolvekamp and Waterman, 1960). We have found evidence supporting such a generalization. At the same time, this evidence suggests that the enlargement of the gill chamber achieves the highest value in groups not showing any folding of the chamber's lining (i.e., *Cardisoma, Gecarcinus, Dilocarcinus*). As such modifications are developed (i.e., *Ocypode*), the branchial chamber volume decreases when the comparisons are made for equal body weight ranges (see Fig. 3). In general, for the species studied there is a gradual loss in volume of the branchial chamber accompanied by increasingly complex chamber walls. Thus, for a given body weight, the largest branchial chambers are those with a simple lining and the smallest, those with a complex lining. When modifications of the gill chamber walls are extreme, as in the "lung" of the Pseudothelphusidae, the ratio of branchial chamber volume to body weight becomes even more reduced. However, for all cases studied, there was an allometric relationship ($\ln y = \ln b + \alpha \ln x$) between the volume of the chamber and the weight of the animal. The volume of the gill chamber related to body weight determines three categories of chamber growth. For these three categories of chamber development, the slopes (α) of the regression lines was found to be similar ($P < 0.01$), indicating that the rate of increment of the gill chamber volume for all three groups was the same, but had a different coefficient, "b." The allometric regressions were empirically determined; they applied only to size ranges represented by the actual data (Gould, 1966). Thus, any interpretation of the coefficient "b" must be made cautiously. However, the initial values shown in the regression curves (Fig. 3) suggest different initial growth rates for each of the three categories.

During the process of land colonization by crabs, new environmental conditions generated new functional problems. In these crabs the problem of respiration was solved in three different ways, corresponding to the three categories of branchial chamber development referred to above. These categories were not apparently related to the habitat where the species normally occur. A simple lining accompanied by a relatively large chamber was found in the Gecarcinidae and Trichodactilidae. The species studied from these two families normally occur in different types of habitats (see Table I). A second category is represented by *O. quadrata*. This species has a lining with folds in a mid-sized branchial chamber. *O. quadrata* is commonly found in the same type of habitat as *G. lateralis*. Thirdly,

high power view of the middle-area of the lining from a transverse section; and e) high power view of the lower layer of the lining which surrounds the chamber's lumen.

FIGURE 11. *Dilocarcinus dentatus* (Randall): a) low power view of the transverse section of the branchial chamber wall; b) high power view of the upper layer of the lining; and c) high power view of the lower layer which surrounds the chamber's lumen.

the most conspicuous lining was presented by the pseudothelphusids which normally inhabit small mountain streams.

It appears that a variety of factors, in addition to change from an aquatic to terrestrial life, have affected the development of respiratory structures. For instance, *Ocypode*, the ghost crab, is well known by its quick runs on open sandy beaches; it has a small and thin carapace. Its nocturnal habitat and fast escape reaction are usually invoked as a mechanisms of defense against predation. With this adaptive strategy, an acceleration of its metabolic rate is to be expected and seems to be confirmed by physiological measurements (Standaert, 1970; Veerannan, 1974; Vernberg, 1956; Vernberg and Vernberg, 1972). The development of a greater surface available for aerial respiration in *Ocypode* may be a response to this need for higher oxygen uptake. *Gecarcinus* is found in the same general habitat, but is larger, has a more durable carapace, and is somewhat less speedy in response. Its adaptive strategy is very different from that of the more elaborate respiratory structures, having evolved towards a large gill chamber with a heavily vascularized lining devoid of folds or protuberances.

It is obvious that behavior has both a physiological and a structural basis; as the function and structure are interrelated, the integrated response of a wide variety of mechanisms is responsible for the survival of any organism. Such an integrated response becomes more evident when a comparison is established in a group of organisms from two or more types of environments, *i.e.*, terrestrial and aquatic crabs.

Pseudothelphusids are very quick in their escape reaction and aggressive behavior; they also have developed relatively strong chelae. However, their body is not necessarily robust. All the species of Pseudothelphusidae studied had the smallest branchial chamber volume in relation to body weight, thus reflecting a relatively small body form. Also, specimens of this family have been found very far from the water or under rocks of creek basins when no water was flowing (Holthuis, 1959). Furthermore, the specimens used in the present work were kept in the laboratory up to two months in containers which had just a thin layer of water. These facts indicate the ability of these organisms to survive in air for prolonged periods of time. Pseudothelphusids represent the group that has the most complex respiratory structure in addition to the branchiae, and, seemingly, the group that has achieved a more efficient use of the space of the gill chamber for respiratory purposes while at the same time remaining relatively small.

In brachyurans, the branchial chamber space becomes filled with gonads before the egg-bearing period. The availability of this space would affect the biomass directly involved in the reproductive process, *i.e.*, clutch size. Thus, the volume of the branchial chamber may be reduced by the presence of large gonads, and accessory structures may be necessary to enhance its respiratory function.

It is concluded that the development of accessory respiratory structures not only is a response to the differential availability of aquatic and aerial oxygen or to the need for oxygen uptake, but also involves a wide variety of interacting factors.

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SUMMARY

1. The structure, form and relative growth of the branchial chamber was studied in several species of amphibious crabs that inhabit marine, estuarine and fresh water. This study revealed a general trend in the solution of the problems of aerial respiration.

2. Growth of the branchial chamber volume is allometric with respect to the body weight of the crab. Data suggest the existence of three categories of complexity in the form, structure, and growth of the branchial chamber of amphibian crabs. A lessening in the chamber's volume is compensated for by an increment in the lining's surface and by development of new structures inside the chamber itself.

3. The shape of the gill chamber and the kind of respiratory structures, in addition to the branchiae, are not directly related to habitat.

4. Histological examination of the gill chamber lining showed that the structure is very similar in its general conformation in all species studied.

5. Species of Pseudothelphusidae from Venezuela mountain creeks present a perforated structure which, by qualitative tests, proved to be a respiratory organ, accessory to the gills. The location of this "lung" is on the anterolateral angle of the ceiling of the branchial chamber. The "lung" is also present in species of Pseudothelphusidae from Central and South America, including species from the Antilles. From a total of fourteen species examined from this family, only a Mexican cave dwelling species, *Potamocarcinus mociñoi*, did not have this structure.

6. The external area, density of perforations, diameter of perforations, and thickness of the respiratory structure found in Pseudothelphusidae were measured; these parameters appear to change with respect to the size of the animal and the species considered. However, the lack of data on individual species does not allow a more definite conclusion about the inter-specific variation.

7. The development of additional respiratory structures are interpreted as being a consequence of a set of environmental conditions new to the organisms as they colonize land, not just a response to the need for aerial respiration.

LITERATURE CITED

BERTOLINI, F., 1933. Sulle funzioni dei polmoni acquatici delle oloturie. *Pubbl. Staz. Zool. Napoli.*, **13** : 1–11.

BERTOLINI, F., 1934. Nuove ricerche sulla funzione respiratoria dei polmoni acquatici delle oloturie. *Arch. Zool. Ital.*, **20** : 579–590.

BLISS, D. E., 1963. The pericardial sacs of terrestrial Brachyura. Pages 59–78 in H. B. Whittington and W. D. I. Rolfe, Eds., *Phylogeny and evolution of Crustacea.* Museum Comparative Zoology. Harvard University, Cambridge, Massachusetts.

BLISS, D. E., 1968. Transition from water to land in decapod Crustacea. *Am. Zool.*, **8** : 355–392.

BORRADAILE, L. A., 1903. Land crustaceans. Pages 64–100 in J. S. Gardiner, Ed., *The fauna*

and geography of the Maldive and Laccadive Archipelagoes, Vol. 5. Cambridge University Press, Cambridge, Massachusetts.

CAMERON, J. N., 1975. Aerial exchange in the terrestrial Brachyura *Gecarcinus lateralis* and *Cardisoma guanhumi. Comp. Biochem. Physiol,* **52A** : 129–134.

CARTER, G. S., 1931. Aquatic and aerial respiration in animals. *Biol. Rev.,* **6** : 1–35.

DENNELL, R., 1960. Integument and exoskeleton. Pages 449–472 in T. H. Waterman, Ed. *Physiology of Crustacea, Vol. I.* Academic Press, New York.

EDNEY, E. B., 1960. Terrestrial adaptations. Pages 369–393 in T. H. Waterman, Ed. *Physiology of Crustacea, Vol. I.* Academic Press, New York.

EDNEY, E. B., AND J. O. SPENCER, 1955. Cutaneous respiration in woodlice. *J. Exp. Biol.,* **32** : 256–269.

GIFFORD, C. A., 1962. Some observations on the general biology of the land crab, *Cardisoma guanhumi* Latreille, in South Florida. *Biol. Bull.,* **123** : 207–223.

GOULD, S. J., 1966. Allometry and size in ontogeny and phylogeny. *Biol. Rev.,* **41** : 587–640.

GRAY, I. E., 1957. A comparative study of the gill area of crabs. *Biol. Bull.,* **112** : 34–42.

HARMS, J. W., 1932. Die realization von Genen und die consecutive adaptation. II. *Birgus latro* L. als landkrebs unseine Bezeihugen zu den Coenobiten. *Z. Wiss. Zool.,* **140** : 167–290.

HERREID, C. F., AND C. A. GIFFORD, 1963. The burrow habitat of the land crab *Cardisoma guanhumi* Latreille. *Ecology,* **44** : 773–775.

HOLTHUIS, L. B., 1959. The Crustacea Decapoda of Suriname (Dutch Guiana). *Zool. Verh. Leiden,* **44** : 1–296.

HUGHES, D. A., 1966. Behavioural and ecological investigations of the crab *Ocypode cerathophthalmus* (Crustacea : Ocypodidae). *J. Zool. London,* **150** : 129–143.

JOBERT, M., 1876. Recherches sur l'appareil respiratoire et le mode de respiration de certaines Crustacés Brachyures (crabes terrestres). *Ann. Sci. Nat. Ser. Zool. Biol. Anim.,* **4** : 1–5.

PEARSE, A. S., 1929. Observations on certain littoral and terrestrial animals at Tortugas, Florida, with special reference to migrations from marine to terrestrial habitats. *Pap. Tortugas Sta., Carnegie Inst. Washington,* **39** : 205–223.

PEARSE, A. S., 1950. *The emigrations of animals from the sea.* Sherwood Press, Dryden, New York, 210 pp.

PEARSE, A. G., 1960. *Theoretical and applied histochemistry.* J. and A. Churchill, Ltd., London, 998 pp.

RODRIGUEZ, G., AND A. E. SMALLEY, 1969. Los cangrejos de agua dulce de México de la familia Pseudothelphusidae (Crustacea, Brachyura). *An. Inst. Biol. Univ. Nac. Autón. Mex. Serv. Cienc. Mar Limnol.,* **40** : 69–112.

SIMPSON, G. G., A. ROE, AND R. C. LEWONTIN, 1960. *Quantitative zoology.* Harcourt, Brace and Co., New York, 440 pp.

STANDAERT, T. A., 1970. Aerial and aquatic respiration of the land crab *Cardisoma guanhumi. Ph.D. Dissertation, Duke University,* Durham, North Carolina, U.S.A., 112 pp.

VALENTE, D., 1948. Mecanismo da respiração de *Trychodactylus petropolitanus* (Goeldi). *Bol. Fac. Filos. Cienc. Let. Univ. Sao Paulo Ser. Zool.,* **13** : 259–316.

VEERANNAN, K. M., 1974. Respiratory metabolism of crabs from marine and estuarine habitats : an interspecific comparison. *Mar. Biol.,* **26** : 35–43.

VERNBERG, F. J., 1956. Study of the oxygen consumption of excised tissue of certain marine decapod crustaceans in relation to habitat. *Physiol. Zool.,* **29** : 227–234.

VERNBERG, W. B., AND F. J. VERNBERG, 1972. *Environmental physiology of marine animals.* Springer-Verlag, New York, 346 pp.

WOLVEKAMP, H. P., AND T. H. WATERMAN, 1960. Respiration. Pages 35–100 in T. H. Waterman, Ed., *The Physiology of Crustacea, Vol. I.* Academic Press, New York.

ZOOND, A., AND E. CHARLES, 1931. Studies in the localization of respiratory exchange in invertebrates. I. The respiratory mechanism of the freshwater crab *Potamonautes. J. Exp. Biol.,* **8** : 250–257.